Soil Biology and Biochemistry 134 (2019) 25–35

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Testing the dependence of microbial growth and carbon use efficiency on nitrogen availability, pH, and organic matter quality

Alex Silva-Sánchez[1], Margarida Soares[1], Johannes Rousk*

Section of Microbial Ecology, Department of Biology, Lund University, Ecology Building, 22362, Lund, Sweden

ARTICLE INFO

Keywords:
Fungal-to-bacterial dominance
Microbial growth efficiency
Detrital food web
Nitrogen fertilisation
Carbon sequestration
Growth-limiting factors

ABSTRACT

Microbial carbon use efficiency (CUE), or the partitioning of assimilated C into growth or respiration, is a key parameter that is central to understanding the soil C cycle and its feedback to environmental and climate change. The availability of nitrogen (N), organic matter (OM) quality and environmental factors influence CUE indirectly by affecting growth rates and respiration of the major microbial decomposers in soil, including fungi and bacteria. In the present study we set out to evaluate the effect of N-additions (mineral N fertiliser), increased pH (lime), and increased OM quality (plant litter addition) on microbial growth, respiration, and resulting CUE. We sampled beech and spruce forest stands each including two levels of soil fertility. In laboratory microcosm experiments we then manipulated the availability of mineral N, pH and OM quality during the course of 60 days and measured rates of bacterial and fungal growth, respiration, and resulting CUE. We observed that growth rates of both bacteria and fungi were stimulated by increased OM quality through litter additions, but when combined with increased pH, the ratio shifted in favour of bacteria, while a shift towards fungal dominance was observed when litter was combined with N additions. Overall bacterial growth was stimulated by increased pH and reduced by addition of mineral N, while fungal growth appeared unaffected by both factors. The ratio of fungal to bacterial growth varied between 0.02 and 0.7, suggesting that 0.4 to 50 times more detrital-C was used by bacteria than by fungi in the dataset. Consistently negative correlations between fungal and bacterial growth suggested competitive interactions during the microbial use of detrital C, with bacteria being the dominant competitor. Estimated levels of microbial CUE ranged from < 0.05 to 0.5, and higher levels of CUE were associated with higher dominance of bacteria in soils with higher pH and lower N availability. Taken together, differences in CUE were linked to the dominance of fungi or bacteria. When bacterial growth was inhibited by mineral N or low pH, a competitive release resulted in a stimulated fungal growth and detrital C-use, which yielded reduced CUEs.

1. Introduction

Soils represent large and sensitive pools of carbon (C) that have a critical role for regulating atmospheric CO_2 concentrations (Denman et al., 2007). Soil fungi and bacteria dominate the decomposer processes that turn over organic matter (OM), and these two major decomposer groups are thought to form the basis of distinct detrital food webs that differ in their ability to sequester C and maintain soil physical structure and plant fertility (Waksman and Starkey, 1924; Le Guillou et al., 2012). For instance, a fungal detrital pathway of resource-use is thought to result in a slower energy turnover and a system less prone to leak nutrients, while a bacterial detrital pathway is thought to result in faster food-web energy fluxes, and a higher rate of production of inorganic nutrients (Wardle et al., 2004). Since in soil, biomass estimates do not reflect microbial process rates (Blagodatskaya and Kuzyakov, 2013; Rousk, 2016), estimates of growth rates are required to capture energy fluxes through detrital food webs. Powerful regulators of the division between fungal and bacterial energy channels within the detrital food web have been shown to include soil pH (Rousk et al., 2009; Fernandez-Calvino and Bååth, 2010), organic matter quality (Strickland et al., 2009; Rousk and Frey, 2015; Kallenbach et al., 2016), and mineral nutrient availabilities (Henriksen and Breland, 1999; Rousk et al., 2011). However, often these factors are confounded in comparisons of different ecosystems along e.g. fertility gradients (Giesler et al., 1998; Rousk et al., 2013; Blasko et al., 2015; Sterkenburg et al., 2015; Kristensen et al., 2018), and only few factorial

* Corresponding author. Microbial Ecology, Department of Biology, Lund University, 22362, Lund, Sweden.
E-mail address: johannes.rousk@biol.lu.se (J. Rousk).
[1] The authors contributed equally, and share first-authorship.

https://doi.org/10.1016/j.soilbio.2019.03.008
Received 28 January 2019; Received in revised form 5 March 2019; Accepted 6 March 2019
Available online 09 March 2019
0038-0717/ © 2019 Elsevier Ltd. All rights reserved.

experimental resolutions of them have been reported to date (Calder and Macleod, 1974; Johnson et al., 2005; Rousk et al., 2011). As such, there is still a need to combine natural fertility gradients with experiments to disentangle causal factors.

When OM is used by the decomposer community, a fraction is metabolised into CO_2 and returned to the atmosphere, while the other fraction is used for microbial growth and forms microbial-C that potentially can be stored in soil (Liang et al., 2017). The ratio of C used for microbial growth to the total use defines the microbial carbon-use efficiency (CUE). As such, the microbial CUE has been identified to be a key parameter that will characterise how ecosystems will feedback to climate change (Allison et al., 2010; Frey et al., 2013; Bradford et al., 2016b). The urgent need to understand how the microbial CUE is regulated in the environment contrasts sharply with our current limited understanding (Bradford et al., 2016a; Geyer et al., 2016). Drawing from the studies of microbial strains in culture, a number of expectations for the important factors that determine microbial CUE have emerged (Roller and Schmidt, 2015). For instance, it is predicted microbial CUE will increase with higher C-substrate quality (Ågren and Bosatta, 1987; Roller and Schmidt, 2015), and will decrease with stronger nutrient limitation (Sterner and Elser, 2002; Manzoni et al., 2012). In addition, there are expectations that fungi and bacterial decomposers in soil have fundamentally different levels of CUE (Six et al., 2006). Therefore, environmental factors that modulate the balance between fungi and bacteria in decomposition, such as soil pH (Rousk et al., 2009), should also be a dominant regulator of CUE. To date, very few assessments of the environmental factors that regulate microbial CUE exist. No reports are available on how soil pH regulates microbial CUEs, and the few observations available for other candidate factors show variable support for the expectations. For instance, while links between higher N availability and microbial CUE have been reported for agricultural soils with different fertiliser regimes (Spohn et al., 2016), large scale surveys of ecosystems with different N availabilities show inconsistent links between microbial CUE and nutrient availabilities (Sinsabaugh et al., 2013; Soares and Rousk, 2019). Moreover, while links between a stronger fungal dominance of decomposition and higher level of soil C storage, suggestive of increased CUEs, have been reported in artificial soils early in soil formation (Kallenbach et al., 2016) and during litter decomposition in agricultural soils (Malik et al., 2016), this relationship does not seem to extend to the comparison of different ecosystems (Soares and Rousk, 2019).

In a recent study, bacterial and fungal growth rates along with estimates for microbial CUEs were assessed for a range of ecosystems, ranging from subarctic forests to temperate agricultural fields, and including wide ranges of SOC-quality (indexed as respiration per SOC; Fierer et al., 2005, 2006), nutrient availability, and soil pH (Soares and Rousk, 2019). Fungal dominance of decomposition was found to be higher in soils with lower C/N, and higher C-quality. Surprisingly, estimates of CUEs were higher in soils with higher C/N and higher C-qualities. These differences resulted in a negative link between dominance of fungi and microbial CUE. However, within ecosystems, relatively higher nutrient availabilities resulted in lower F/B and higher CUE. The seemingly contradictive patterns found within and between ecosystem call for factorial experiments that can assign causality between the regulating environmental factors for the fungal vs. bacterial dominance of decomposition and the microbial CUE.

In this study, we set out to evaluate the environmental factors that control the fungal-to-bacterial dominance of decomposition and the factors that control microbial CUEs. To accomplish this, we collected samples from beech and spruce forests from high and low fertility sites resulting from groundwater recharge. Higher fertility was associated with both higher availability of the plant limiting nutrient N and higher pH. These samples were then exposed to adjustments of soil pH (liming, increasing soil pH by approx. 1.5 units), mineral N fertilisation (equivalent to 50 kg N ha^{-1} as NH_4NO_3), and OM quality (birch litter addition) in a set of microcosms monitored during 2 months. We hypothesized that the fungal-to-bacterial growth ratio would decrease with higher pH (Rousk et al., 2009) and with higher mineral N (de Vries et al., 2006; Rousk et al., 2011) and would increase with higher OM quality (Rousk and Frey, 2015; Soares and Rousk, 2019). We also hypothesized that microbial CUE would decrease with lower pH (driven by higher fungal dominance), and would increase with both higher mineral N availability (driven by the reduced microbial nutrient limitation) and higher OM quality (driven by higher substrate quality). We expected these links to environmental factors to be consistent within the compared sites, and also in response to the experimental adjustments within sites.

2. Materials and methods

2.1. Soil sampling and experimental design

Soil samples were collected within the experimental forests kept at Öved kloster, in South Sweden (55.401 N, 13.384 E) in late February 2018, after thaw and during frost free conditions. The soil of the area is a Cambisol (WRB, 2015), and the area was historically used for cropped and tilled agriculture. The area includes a groundwater recharge and discharge area, where base-cations are leached from upslope soils which grow infertile and acidic, and then are deposited in discharge areas downslope, which are richer in base-cations are less acidic. Scattered within this area are planted monoculture forest stands of beech and spruce (established during the first half of the 20th century), with birch establishing in patches resulting from tree fall. Three replicate patches (25 m by 25 m) were established, at least 500 m apart, within each of two sets (low fertility in the groundwater recharge area, and high fertility in the groundwater discharge area) of spruce and beech forest stands. This resulted in four treatments (beech low fertility, beech high fertility, spruce low fertility, and spruce high fertility) that were replicated in three (n = 3). In each replicate patch, a composite sample was formed from at least 10 samples cut with a spade of the organic horizon (ranging in thickness between 5 and 15 cm), excluding both litter and mineral soil. The 10 samples were sieved (4 mm) and gently homogenized into plastic bags, and brought into the laboratory. Soil samples were then stored at 5 °C until used in experiments, and the first assessments (described below) started within 5 days of sampling.

The sampled soils were subdivided into eight 100 g samples in 250 ml microcosms with lids (Thermo Scientific™ Nalgene™ Wide-Mouth Straight-Sided PPCO Jars with Closure, 250 mL). The treatments were additions of dried (65 °C under a fan during until constant weight) and ground birch leaf litter (c. 20 mg litter-C g^{-1} soil; a litter found in all studied sites), lime ($CaCO_3$, 10 mg g^{-1} soil; resulting in ca 1.5 units increase in pH in pilot experiments), mineral fertiliser (1.80 mg $(NH_4)_2SO_4$ g^{-1} soil; corresponding to ca 50 kg N ha^{-1}) and were added in a factorial design, resulting in 8 microcosms per replicate and treatment or a total of 96 microcosms. Henceforth, within each of the four sites, these treatments will be referred to using the terms “No” for no litter addition, and “Litter” for the litter addition, along with the treatment terms control: “Co”, N addition: “N”, liming: “pH”, and combined liming and N addition: “N + pH”. The three replicates were run in blocks, offset by ca 2 weeks, to ensure that the experiment could be handled logistically. In total, each block was monitored for c. 2 months, incubated at a controlled 16 °C (± 1 °C), which is a typical summer soil temperature for the area, and at a moisture content corresponding to approximately 50% of the water-holding capacity of each soil (Table 1). Between sampling points, soil moisture was monitored gravimetrically, but adjustments were not necessary (consistently < 0.3 g water loss per 100 g microcosm over 2 months). The microcosms were subsampled for assessments of fungal growth, bacterial growth, and respiration at 10 time-points throughout the 2 months incubations (see later section; on days 1, 2, 4, 8, 12, 17, 24, 33, 46 and 59). In a subset of 4 of these time-points (on days 1, 12, 33 and 59), soil pH and electrical conductivities were also measured (see below).

2.2. Soil chemistry

Soil water content was measured gravimetrically after drying at 105 °C overnight, soil organic matter content was measured as loss on ignition (600 °C overnight). Soil pH and electrical conductivity (EC) were measured in water extracts (1:5, w:V) with a pH or electrical conductivity electrode following a 2 h extraction, and 1 h sedimentation. Soil C and N were analysed using the Dumas dry combustion using an elemental analyser (VarioMAX CN, Elementar, Hanau, Germany).

2.3. Soil microbial parameters

Bacterial growth was estimated using methyl-^{3}H thymidine incorporation into bacterial DNA (Bååth, 1992; Bååth et al., 2001). For this, 1 g of fresh soil was mixed with 20 ml of distilled water, vortexed for 3 min, centrifuged (10 min at 1000 × *g*) and the resulting bacterial suspension was incubated at 16 °C for 2 h with 100 nM thymidine (37 MBq mL^{-1}; Perkin Elmer, Waltham, Massachusetts, USA). The bacteria were killed after 2 h by adding 75 μL of 100% trichloroacetic acid. Centrifugation and washing were performed as described by Bååth et al.(2001). Scintillation cocktail (Ultima Gold, PerkinElmer, Waltham, MA, USA) was added, and the radioactivity was measured using a liquid scintillation counter (TriCarb 2910 TR, Perkin Elmer, UK). The amount of thymidine incorporated into the extracted bacteria (pmol incorporated g^{-1} of soil h^{-1}) was used as a measure of bacterial growth. The incorporated thymidine was converted to bacterial-C production g^{-1} h^{-1} based on the relationship established for litter colonizing bacteria previously applied in soil (Soares and Rousk, 2019).

Fungal growth was measured by the acetate incorporation into ergosterol (Ac-in-erg) method (Newell and Fallon, 1991) adapted for soil (Bååth, 2001; Rousk et al., 2009). Fresh soil (0.5 g) was transferred into test tubes to which 20 μL of ^{14}C-acetate solution ([1–^{14}C] acetic acid, sodium salt, 2.07 GBq mmol^{-1}, Perkin Elmer, UK) and unlabelled sodium acetate were added, resulting in a final acetate concentration of 220 μM in the soil slurry. The slurry was incubated for 4 h at 16 °C before growth was terminated with formalin. Ergosterol was extracted, quantified and separated as previously described by (Rousk and Bååth, 2007a). The ergosterol fraction of each sample was then separated on HPLC equipped with a fraction collector as previously described (Rousk and Bååth, 2007b), mixed with 3 ml of scintillation cocktail, and ^{14}C derived radioactivity was measured using liquid scintillation (see above). The amount of acetate incorporated into ergosterol (pmol h^{-1} g^{-1} soil) was used as a proxy for fungal growth (pmol Ac g^{-1} h^{-1}), and the concentration of ergosterol was used to estimate fungal biomass-C, assuming a fungal ergosterol content of 11 mg g^{-1} fungal biomass-C (Jörgensen, 2000; Rousk et al., 2009). The incorporated Ac was converted to fungal-C production g^{-1} h^{-1} based on the relationship established for litter colonizing fungi previously applied in soil (Soares and Rousk, 2019).

Basal soil respiration (1 g) was determined as the accumulation of CO_2 in 20 ml glass vials during 2–6 h incubation (depending on the rate at sampling) at 16 °C in the dark using a gas chromatograph equipped with a methanizer and a flame ionization detector (YL6500 GC, YL Instruments, Gyeonggi-do, Korea).

2.4. Statistics and calculations

Values of cumulative bacterial growth, fungal growth, and respiration were estimated as the integral of rates over time. The total microbial growth was estimated as the sum of fungal and bacterial growth. CUE was estimated as the ratio between total microbial growth to the total microbial carbon use (total microbial growth + respiration). Microbial CUEs were estimated both as rates over the time-course of the full microcosm experiments, and as the "resulting microbial carbon use", i.e. ratio of cumulative microbial growth to the cumulative total carbon use (cumulative microbial growth + cumulative respiration).

There were interactions between treatments and sites and the time-factor for all studied rates (Figs. 1–4), making the use of repeated measures ANOVAs inappropriate. Instead, treatment and site differences were tested on the cumulative values of bacterial growth, respiration, and CUEs, with 3-way ANOVAs, considering the factors 'treatment' (control, pH, N, and N + pH), 'litter' (no, litter) and 'site' (poor spruce, rich spruce, poor beech or rich beech) and in all factorial combinations. To meet the assumptions of the ANOVA analyses, values were log-transformed before analysis. For factors with more than 2 levels, Tukey's HSD pair-wise comparisons were used to compare treatments with an $\alpha = 0.05$.

3. Results

3.1. Soil physiochemistry

The soil organic matter contents in freshly sampled soils were lower in the two beech sites (poor beech: 27 ± 3.9%; rich beech: 22 ± 2.7%) than it the two spruce sites (poor spruce: 41 ± 1.3%; rich spruce: 45 ± 1.7%), while fertility levels did not affect it. In the freshly sampled soils, soil pH was marginally lower ($P = 0.03$) in the two beech sites (poor beech: 4.5 ± 0.08; rich beech: 4.8 ± 0.05) than it was in the spruce sites (poor spruce: 4.8 ± 0.10; rich spruce: 5.2 ± 0.20), with a marginally higher pH in the higher fertility site within each set ($P = 0.02$) with no interaction between forest type and fertility level ($P = 0.83$). When subjected to the microcosm treatments, soil pH ($P < 0.001$) and electrical conductivity ($P < 0.001$) were affected by both treatment and site without any interaction between the factors. Tukey's tests pair-wise comparisons showed that the pHs of all three sites were distinguishable, ranging from low to high, as poor spruce, rich spruce, poor beech, and rich beech (Table 1). The treatments also systematically affected pH, ranging from lowest to highest as "N" < "Co" < "pH + N" < "pH" (Table 1). Electrical conductivities (EC) aligned similarly among the sites, although not all subsets were distinguishable, from lowest in the poor spruce, poor beech and rich beech intermediate, and rich spruce highest (Table 1). The treatments resulted in highest ECs in the combined pH + N, intermediate levels in the separate N and pH treatments, while the lower levels were found in the treatments without N or pH amendments (Table 1).

3.2. Soil microbial growth, respiration and carbon-use efficiencies (CUEs)

Overall bacterial growth rates were distinguishable between the four sites, although differences were small, with marginally higher rates occurring in beech forests than in spruce (Fig. 1). Bacterial growth was similarly stimulated in both treatments that increased soil pH (pH, and pH + N) in all 4 sites, resulting in rates that peaked around day 4, and then continually decreased over time. Bacterial growth rates were higher in treatments with litter than those without. In contrast, N additions universally decreased rates of bacterial growth (Fig. 1). These responses and differences resulted in significant effects in the cumulative bacterial growth by all three factors 'site', 'litter', and 'treatment' (all $P < 0.001$; Fig. 1), but without interactions between them. The highest overall cumulative bacterial growth resulted in the poor beech site, and the lowest in poor spruce, with the two others intermediate and not clearly distinguishable. Litter resulted in nearly double the amount of bacterial growth compared to no litter, while the four treatments were all distinguishable, ranked from lower to highest cumulative bacterial growth as N < Co < pH + N < pH (Fig. 1).

Differences in fungal growth rates were small but overall distinguishable between sites, with slightly higher rates in beech compared with spruce forests (Fig. 2). In contrast with bacterial growth, the treatments that increased pH both resulted in reduced fungal growth rates, while the N addition treatments stimulated rates of fungal growth (Fig. 2). The responses to treatments seemed to grow stronger when combined with litter compared to without (Fig. 2), and like bacteria,

Table 1
Soil characteristics for the different soil sites and treatments. Soil sites were characterised based on dominating tree species on the forest stand and fertility level (see materials and methods). Values are means with standard error (SE).

Site	Treatment	Soil pH		Electrical conductivity (μS cm^{-1})		Water content (% of dry mass)	
		Mean	SE	mean	SE	mean	SE
Poor spruce	No − Co	4.2	0.18	139	7.6	108.0	10.0
	No - pH	5.8	0.17	261	51.5	104.0	9.4
	No − N	4	0.1	607	55.8	110.8	9.4
	No - pH + N	5.6	0.2	776	62.7	107.6	12.1
	L - Co	4.5	0.03	103	2	100.5	5.5
	L - pH	6.1	0.2	161	28.4	105.5	10.5
	L - N	4.3	0.14	636	12.1	104.9	8.9
	L - pH + N	5.7	0.18	637	54.9	103.0	8.9
Rich Spruce	No − Co	4.5	0.18	175	8.9	122.6	2.4
	No - pH	5.9	0.08	348	17	122.3	4.4
	No − N	4.4	0.08	697	9	127.7	2.2
	No - pH + N	5.4	0.18	1032	10	128.9	1.8
	L - Co	4.8	0.08	256	162.6	117.0	4.5
	L - pH	6.3	0.11	257	52.3	121.8	3.0
	L - N	4.5	0.08	658	20	124.2	2.6
	L - pH + N	5.9	0.07	775	16.5	124.2	1.1
Poor Beech	No − Co	4.6	0.12	103	18.5	119.0	14.2
	No - pH	6.4	0.14	316	36.6	115.5	17.4
	No − N	4.4	0.02	673	31.9	118.5	12.2
	No - pH + N	6.2	0.13	940	98.5	108.9	16.6
	L - Co	5.3	0.11	86	1.3	114.6	18.2
	L - pH	6.6	0.05	220	30.2	107.5	13.9
	L - N	4.5	0.11	614	26.3	111.3	12.5
	L - pH + N	6.2	0.18	630	120.7	108.9	11.3
Rich Beech	No − Co	5.2	0.05	99	15.8	84.3	10.3
	No - pH	6.7	0.06	396	30.1	95.0	11.8
	No − N	5.1	0.05	612	27.1	92.9	12.0
	No - pH + N	6.4	0.1	922	10	88.3	10.5
	L - Co	5.6	0.05	85	7.5	91.0	12.7
	L - pH	6.9	0.04	245	39.3	84.8	8.0
	L - N	5.1	0.06	551	28.8	80.8	6.0
	L - pH + N	6.6	0.04	773	66.7	83.6	12.0

differences between treatments along with maximal rates appeared to peak around day 4 (Fig. 2). These responses and differences resulted in significant effects on cumulative fungal growth by each of the three factors (Fig. 2, all $P < 0.001$), but without interactions excepting the noted stronger response by treatments when combined with litter ($P < 0.0001$). Cumulative fungal growth was highest in rich beech, and lowest in rich spruce, with the two other sites indistinguishable at intermediate levels (Fig. 2), overall about twice the levels when combined with litter compared to without, and ranked from low to high values according to pH + N = pH < Co < N, with about twice the cumulative values in N compared with the pH-treatments (Fig. 2).

When considering the fungal and bacterial growth rates together, as the fungal-to-bacterial growth ratio, the similarities that the two groups showed between sites, and in response to litter, resulted in no significant effect by site ($P = 0.32$) and litter ($P = 0.10$) on the fungal-to-bacterial growth ratio (Figs. 1 and 2). However, the strikingly different responses by the two decomposer groups to pH and N treatments (see above) resulted in a highly significant effect by treatment ($P < 0.0001$), resulting in fungal-to-bacterial growth ratios ranging from about 0.02 (pH treatment in rich spruce) to 0.7 (N treatment in poor beech). As such, between 0.4 and 50 times more C was used by bacteria than by fungi in the dataset. Overall, the treatments ranged from lowest ratio in the pH and pH + N treatments of less than 0.05, an intermediate level in the control treatment of about 0.2, to the highest level in the N treatment, reaching values of 0.7.

Respiration rates varied among the sites, with overall higher rates occurring in the beech compared to the spruce sites (Fig. 3). Rates peaked immediately, and then decreased over time in all sites, treatments, and litter additions (Fig. 3). Litter additions consistently increased rates throughout the experiment, and both treatments that increased pH resulted in elevated rates, while the N addition marginally reduced rates unless combined with litter (Fig. 3). These differences and responses resulted in strong significant effects on cumulative respiration by all three factors (all $P < 0.0001$), but without other interactions than a stronger treatment effect when combined with litter ($P = 0.0018$). The levels of cumulative respiration were about 50 (in poor) or 100% (in rich) higher in beech than spruce forests, increased by more than a factor of 2 when combined with litter, and ranged from low to high cumulative respiration according to N < Co < pH + N < pH, with a factor two difference between the extremes (Fig. 3).

The microbial CUE was dynamic over time, and ranged more than 10-fold from transiently high values marginally exceeding 0.5, to values lower than 0.05 (Fig. 4). There were small but distinguishable differences between sites, with highest overall values in rich spruce and lowest in rich beech (Fig. 4). The addition of litter only marginally increased overall rates of CUE, while the treatments that increased soil pH affected rates of CUE the most, and with the largest dynamics over time (Fig. 4). These differences and responses resulted in significant effects by all three factors on the resulting microbial CUEs during the 2 months of study (treatment and site both $P < 0.0001$, litter $P = 0.01$), where levels were lowest in the rich beech and about 50% higher in the rich spruce, with the two other sites being intermediate. Litter addition only marginally increased the resulting CUE, while the treatments were all clearly distinguishable, ranging from lowest to highest CUE level as N < Co < pH + N < pH, and with values diverging up to a factor of three (Fig. 4). The only significant interaction was between treatment and site, where the effect size of the responses of the treatments varied between sites (Fig. 4).

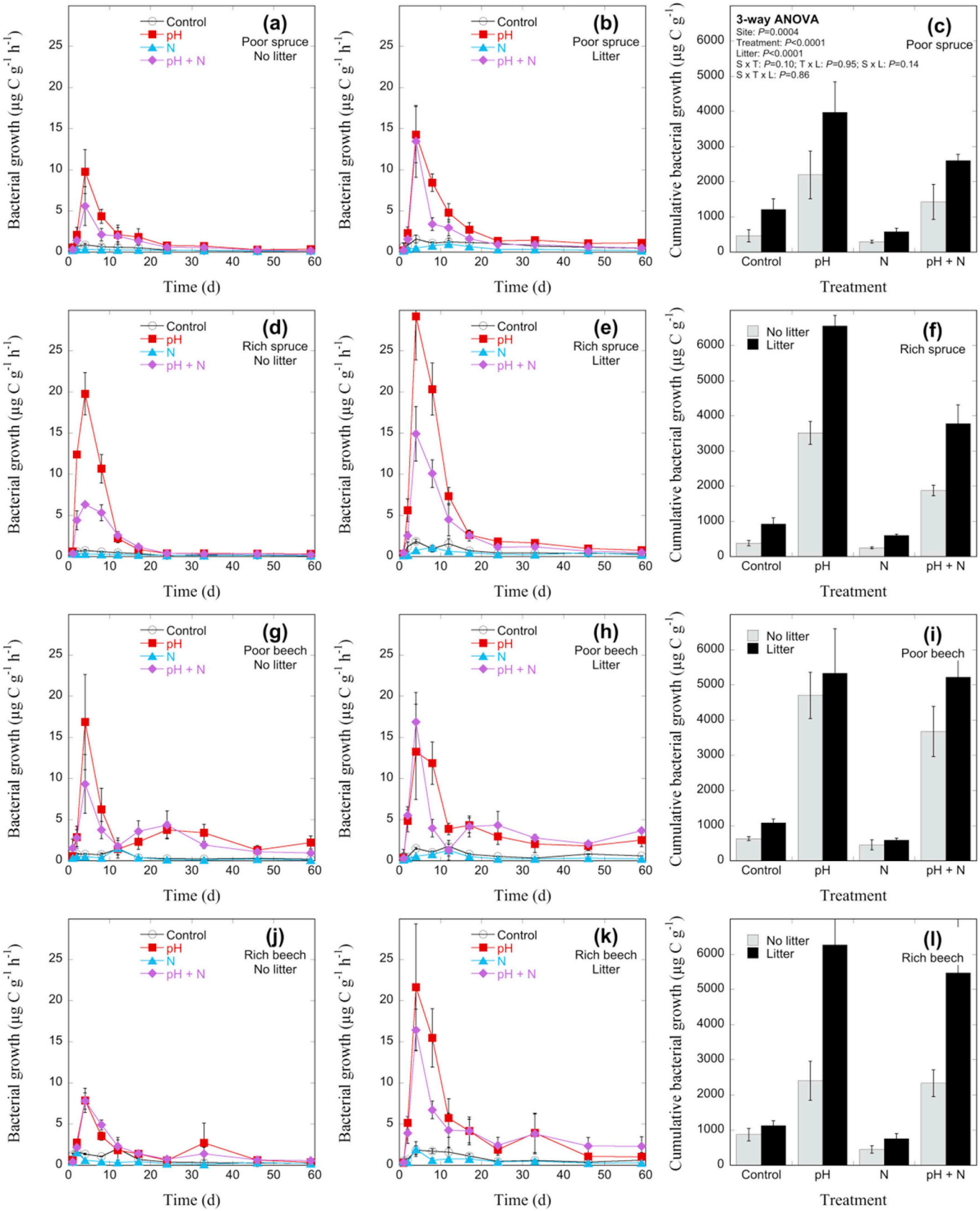

Fig. 1. Bacterial growth rates without litter additions (left-hand panels) and with litter additions (centre panels), and bacterial cumulative growth (right-hand panels) in spruce forest stands (panels a–f) and beech forest stands (panels g–l). Data points show mean values ± 1 SE; for some data points the error bars are smaller than the symbol itself.

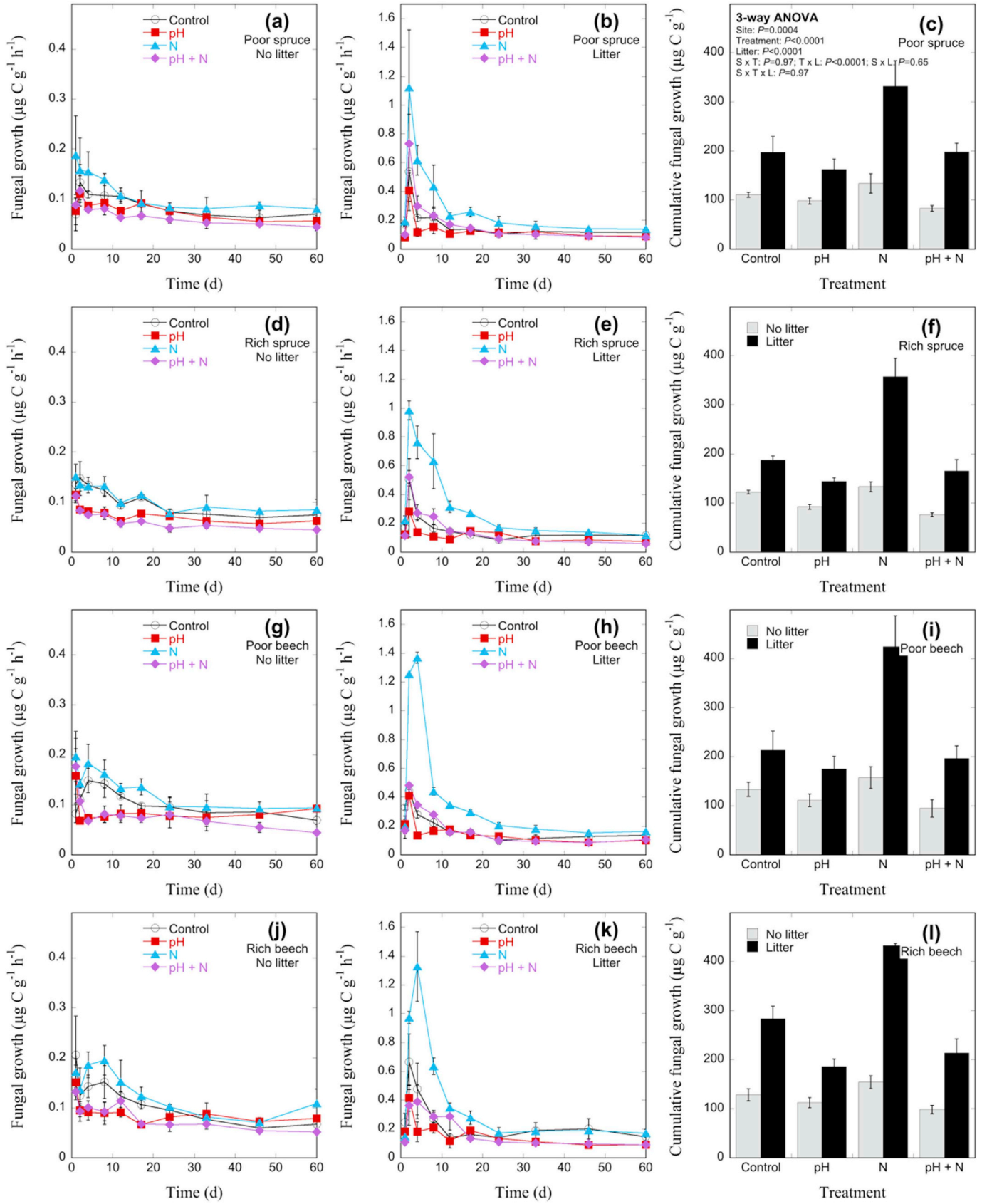

Fig. 2. Fungal growth rates without litter additions (left-hand panels) and with litter additions (centre panels), and fungal cumulative growth (right-hand panels) in spruce forest stands (panels a–f) and beech forest stands (panels g–l). Data points show mean values ± 1 SE; for some data points the error bars are smaller than the symbol itself.

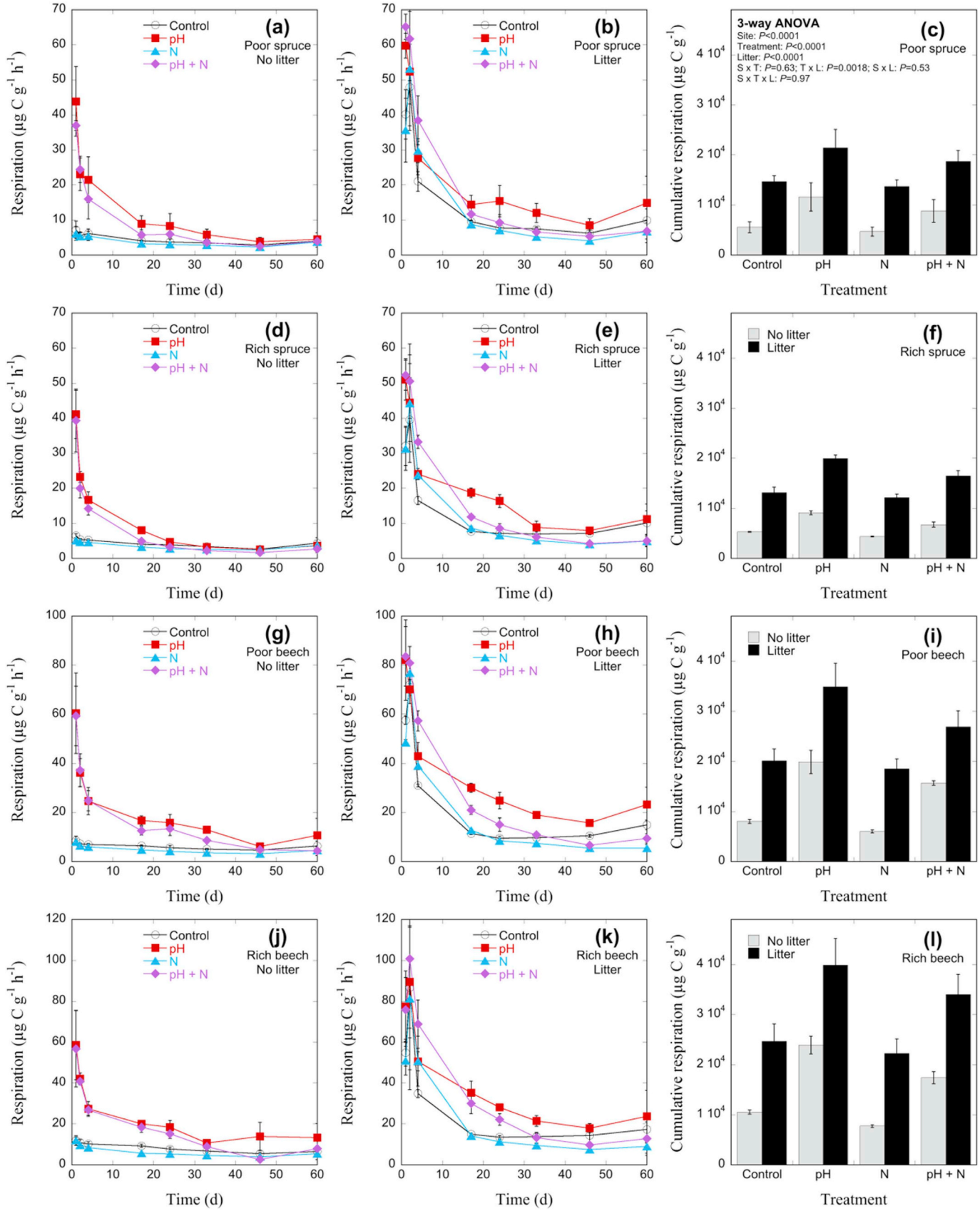

Fig. 3. Respiration rates without litter additions (left-hand panels) and with litter additions (centre panels), and cumulative respiration (right-hand panels) in spruce forest stands (panels a–f) and beech forest stands (panels g–l). Data points show mean values ± 1 SE; for some data points the error bars are smaller than the symbol itself.

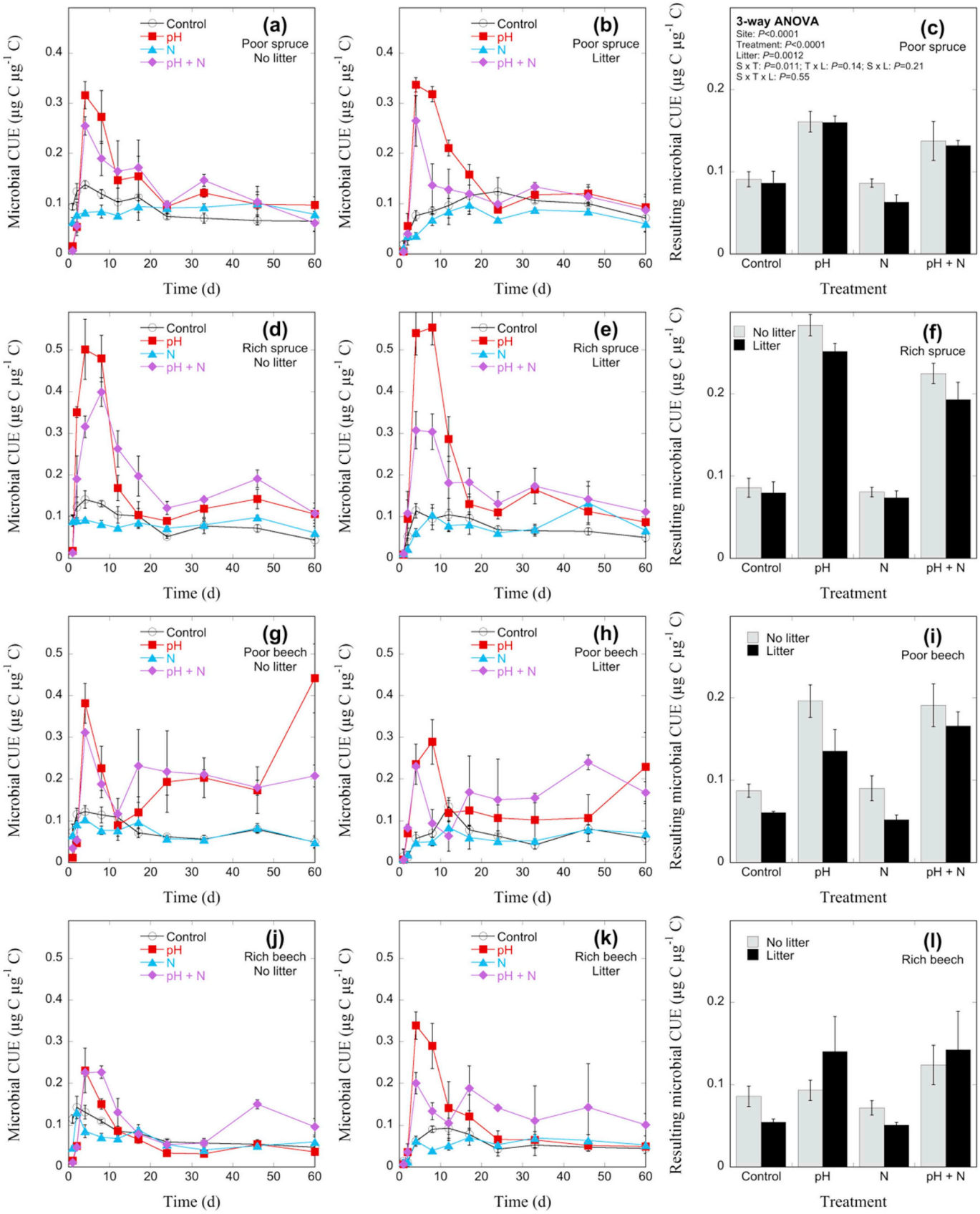

Fig. 4. Microbial carbon use efficiency without litter additions (left-hand panels) and with litter additions (centre panels), and resulting microbial carbon use efficiency (right-hand panels) in spruce forest stands (panels a–f) and beech forest stands (panels g–l). Data points show mean values ± 1 SE; for some data points the error bars are smaller than the symbol itself.

4. Discussion

4.1. Site differences and experimental treatments

In this study, we set out to evaluate the environmental factors that (i) control the fungal-to-bacterial dominance of decomposition, by resolving the fraction of organic matter used by fungi compared to that used by bacteria, and (ii) those that control microbial CUEs. Our power to resolve and compare these factors rested on our ability to include a relevant range of differences among the studied sites, and induced by our treatment factors. The soils assessed included two types of forest stands (beech and spruce) that each included two levels of fertility (poor and rich) due to groundwater recharge and discharge differences. As anticipated, the rich sites consistently had higher pHs, and electrical conductivity levels, presumably capturing differences due to variable concentrations of mineral nutrient salts, which were higher in rich spruce sites, but not so in beech (Table 1). In addition, respiration per SOM is often used as a proxy for C-quality (Fierer et al., 2005, 2006), which is another metric thought to systematically vary with site fertility (Wardle et al., 2004), and this value increased from rich to poor sites in beech, but not in spruce (Table 1). However, overall, we did include a range of soil pHs from 4.1 to 5.1, an almost 2-fold range in mineral nutrient concentrations (Table 1), and a 2-fold range of SOM quality differences within the four replicated sites considered (Fig. 3). As such, the chosen sites did present us with a range of soil fertilities to consider comparable from ranges measured on earlier work on fertility gradients (Giesler et al., 1998; Blasko et al., 2015; Sterkenburg et al., 2015; Kristensen et al., 2018). More importantly than initial differences between the four sites, the manipulative treatments experimentally induced ranges of pHs from 4.0 to 6.7, 10-fold ranges of electrical conductivities, reflecting large differences in nutrient salt concentrations, and nearly 10-fold ranges in SOM quality, as indexed by respiration per SOM (Fig. 3). Therefore, the combination of a range of field sites were factorially exposed to adjustments of three factors of soil fertility (soil pH, N availability, and SOM quality). The factorial experimental design would also allow us to discriminate between them. As such, we could address the posed hypotheses with the power intended in the study design.

4.2. The environmental controls of the relative dominance of fungi-to-bacteria

We hypothesized that the fungal-to-bacterial growth ratio would decrease with higher pH, with higher mineral N, and with higher OM quality. Although both fungal growth and bacterial growth differed among the sites (Figs. 1 and 2), these differences were positively correlated, such that the ratio between fungi and bacteria remained unchanged. However, when pH was increased, the fungal-to-bacterial growth ratio decreased substantially, both when combined with N or not. These decreases of the ratio were driven by both lowered fungal growth rates (Fig. 2), and stimulated bacterial rates (Fig. 1). These patterns were anticipated and are consistent with previously noted relationships between the fungal-to-bacterial growth ratio due to long-term differences in soil pH in a variety of soil types (Rousk et al., 2009, 2011; Fernandez-Calvino and Bååth, 2010), and in response to short-term adjustments of soil pHs (Cruz-Paredes et al., 2017). In concert with this shift toward a lower fungal-to-bacterial growth ratio, an increase in soil pH also increased respiration. It is well known that the availability of soil C is stimulated by a higher pH (Andersson et al., 1994; Jones and Willett, 2006). Therefore, it is possible that both the increased microbial substrate level, direct effects by pH, or a combination of these effects, could have caused these microbial responses.

We also hypothesized that the fungal-to-bacterial growth ratio would decrease with higher mineral N concentrations. In contrast with the predictions following from the presumed higher nitrogen need required for bacterial than for fungal growth (Sterner and Elser, 2002; Strickland and Rousk, 2010), N additions decreased bacterial growth (Fig. 1), and stimulated fungal growth (Fig. 2); responses that reinforced each other and resulted in a pronounced increase in the fungal-to-bacterial growth ratio. Although surprising from a stoichiometric perspective, the short-term inhibition of bacterial growth induced by mineral N additions has been previously found in a range of soils (Aldén et al., 2001; Kristensen et al., 2018; Rosinger et al., 2019). Longer-term responses of fungal-to-bacterial growth to input of N fertiliser appears to be limited if they exist at all (Rousk et al., 2011), however, while the biomass ratio between the group tends to suggest a stronger decrease of fungal than bacterial biomass (de Vries et al., 2006; Rousk et al., 2011). In addition to reducing bacterial growth, the addition of mineral N consistently reduced respiration, which is consistent with the noted N-inhibition of microbial decomposer activity in soil (Fog, 1988; DeForest et al., 2004; Ramirez et al., 2010), the causes for which remain unresolved (although see (Whalen et al., 2018)).

Finally, we hypothesized that the higher OM quality induced by litter additions would decrease the fungal-to-bacterial growth ratio. The microbial response to the increased OM quality induced by litter was more complex than hypothesized, through interactions with the other treatments. The growth rates of both groups were stimulated by litter addition, but when combined with increased pH, the ratio shifted in favour of bacteria, while when combined with N additions, it shifted in favour of fungi. Although the generally held expectation is that there is a link between the lability of C and a lower dominance of fungi of soil C decomposition (Wardle et al., 2004; de Boer et al., 2005; Bardgett and van der Putten, 2014), it is also found that increased litter input into soil, increasing the lability of C, is associated with an increase in the fungal-to-bacterial growth ratio (Rousk and Frey, 2015).

4.3. Fungal-bacterial interaction and the control of decomposition

The negative correlation between fungal and bacterial growth responses to the experimental treatments suggests a negative interaction between the two groups. It prompts the question of how this negative relationship is regulated, which of the groups is the superior competitor and whether this group is compromised or stimulated by the experimental treatments. Earlier work resolving the interaction between the two groups using selective bacterial inhibitors has shown that the removal of bacteria induces large increases in fungal growth responses (Rousk et al., 2008). In addition, across large differences in fungal:bacterial growth ratios driven by differences in pH, the selective suppression of bacteria across the gradient enabled high fungal growth at all pHs, suggesting the negative correlation between fungal growth (and especially the fungal:bacterial growth ratio) and pH was due to bacterial suppression of fungal growth where bacteria were not limited by acid conditions (Rousk et al., 2010). These suggestions for bacteria being the superior competitor are consistent with the obtained results. In response to increasing pH, bacteria increased about 5-fold, resulting in a microbial C-use increase of 3000–6000 $\mu g\ C\ g^{-1}$ (Fig. 1). In response to this change, fungal growth only decreased by around 10%, corresponding to a reduction of $< 100\ \mu g\ C\ g^{-1}$ (Fig. 2). These results suggested that the pH increase released large amounts of new substrate that were nearly entirely used up by bacteria, leaving no substrate to exploit for fungi. In response to mineral N, bacteria were reduced by, 20–50%, resulting in a few hundred $\mu g\ C\ g^{-1}$ lower resource use by bacteria, which coincided with similar levels of increased C-use by fungi (Fig. 2). These results are consistent with a bacterial dominance of the microbial C-resource use, which then can be freed for fungal-use only when bacteria are limited by changed environmental conditions, including acid pHs and high mineral N concentrations.

4.4. The environmental controls of the microbial CUE

While estimates of microbial CUE in environmental samples suggest that they can cover very wide ranges (0.05–0.60) (del Giorgio and Cole,

1998; Manzoni et al., 2012), estimates for soil systems up until recently suggested high values (0.30–0.55) (Sinsabaugh et al., 2013), even with many reports close to the theoretical maximum (0.6–0.8) (Dijkstra et al., 2011; Frey et al., 2013). However, recent reports based on substrate independent methods have revised these estimates, suggesting that average *in situ* soil microbial CUE fall within the range of 0.2–0.3 (Spohn et al., 2016; Walker et al., 2018; Geyer et al., 2019; Zheng et al., 2019). In a recent survey of how microbial CUEs varied in 33 different soils including 9 different sites using the same approaches here reported, a range of microbial CUE in freshly sampled soil samples fell within the lower bound of these values, at 0.03–0.30 (Soares and Rousk, 2019). We can here extend this insight by resolving how CUE developed during 2-month long microcosm incubations with various treatments, yielding a range of CUE from 0.03 to values higher than 0.5, but with most values falling within the range of 0.1–0.3 (Fig. 4). Our study also highlights how temporally dynamic microbial CUEs are, emphasizing the need to better constrain the influence on CUE by environmental controllers that are prone to vary over time in natural soil environments, such as moisture availability, C and nutrient availabilities, and temperature.

In the Soares and Rousk (2019) survey of sites, the strongest predictors that drove the observed variation in CUE estimates among sites were identified to be the fungal-to-bacterial growth dominance, and the availability of N. However, to causally link these putative regulating factors for microbial CUE, experimental verification is needed. Therefore, we hypothesized that microbial CUE would respond to a change in pH (driven by the change of fungal-to-bacterial growth), and would increase with both increased mineral N availability and higher induced OM quality (via shifts in the fungal-to-bacterial dominance). Our experiments demonstrate that higher pH will increase microbial CUE, that higher N availabilities reduce them, while increased OM quality had no consistent effects (Fig. 4). Combining the survey of Soares and Rousk (2019) with the factorial experiment conducted in this study yields useful insights. The links between soil N availability and microbial CUE observed in the survey of sites is not supported by an experimental verification, suggesting that the link to nutrient availability is indirect, presumably driven via the plant community's productivity and thus the microbial C-supply. In addition, the strong link to the relative dominance of C-use by fungi and bacteria uncovered in the survey of sites (Soares and Rousk, 2019) held true in our experimental verification both when the fungal-bacterial growth ratios were adjusted with N or soil pH (Fig. 4). This emphasizes the powerful control that the characteristics of the microbial community has on the ability of soil to accumulate or loose C (Liang et al., 2017). If these results can be extrapolated, the key to controlling C-stocks in soil are management factors that determine the fungal-to-bacterial growth ratio. These suggestions call for a careful and systematic consideration for how the land-use factors that affect fungal dominance in soil will impact the microbial CUE, including e.g. land-use intensity (Bardgett and McAlister, 1999), tillage (Jansa et al., 2003), OM-treatments (Lucas et al., 2014), liming practices (Cruz-Paredes et al., 2017), and the management of plant communities (Lange et al., 2015).

5. Conclusions

In our microcosm study we observed that F:B growth ratios were mostly affected by mineral N availability and pH, with high F:B growth ratios induced by N additions and low F:B ratios induced by high pHs. We observed that higher CUE values resulted in bacterial-dominated systems with higher pH and low N availability. Taken together, our results suggest that elevated pH increased OM availability and thus provided resources that were rapidly used by bacteria and resulted in increased CUE at low F:B ratios. Further, bacteria dominated the use of these freed resources in soil with increased pH by outcompeting fungi for resource use. Only in situations of both N fertilisation and high OM input were fungi stimulated but without changes in CUE. By evaluating both the natural fertility gradients and manipulative microcosm experiments in concert we can conclude that the microbial CUE was not affected by N availability directly. Rather, environmental variation in microbial CUE appears to be determined by variation in the fungal-to-bacterial dominance of detrital C-use, which in turn is a function of soil pH and plant C-input.

Acknowledgements

This work was supported by grants from the Swedish Research Council Formas (grant no. 2018-01315), the Swedish Research Council Vetenskapsrådet (grant no. 2015-04942), and the Knut and Alice Wallenberg Foundation (grant no. KAW 2017.0171). The research is a contribution to the strategic research area Biodiversity and Ecosystems in a Changing Climate (BECC) at Lund University.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2019.03.008.

References

Ågren, G.I., Bosatta, E., 1987. Theoretical-Analysis of the long-term dynamics of carbon and nitrogen in soils. Ecology 68, 1181–1189.
Aldén, L., Demoling, F., Bååth, E., 2001. Rapid method of determining factors limiting bacterial growth in soil. Applied and Environmental Microbiology 67, 1830–1838.
Allison, S.D., Wallenstein, M.D., Bradford, M.A., 2010. Soil-carbon response to warming dependent on microbial physiology. Nature Geoscience 3, 336–340.
Andersson, S., Valeur, I., Nilsson, I., 1994. Influence of lime on soil respiration, leaching of doc, and C/S relationships in the mor humus of a haplic podsol. Environment International 20, 81–88.
Bååth, E., 1992. Thymidine incorporation into macromolecules of bacteria extracted from soil by homogenization centrifugation. Soil Biology and Biochemistry 24, 1157–1165.
Bååth, E., 2001. Estimation of fungal growth rates in soil using C-14-acetate incorporation into ergosterol. Soil Biology and Biochemistry 33, 2011–2018.
Bååth, E., Pettersson, M., Soderberg, K.H., 2001. Adaptation of a rapid and economical microcentrifugation method to measure thymidine and leucine incorporation by soil bacteria. Soil Biology and Biochemistry 33, 1571–1574.
Bardgett, R.D., McAlister, E., 1999. The measurement of soil fungal : bacterial biomass ratios as an indicator of ecosystem self-regulation in temperate meadow grasslands. Biology and Fertility of Soils 29, 282–290.
Bardgett, R.D., van der Putten, W.H., 2014. Belowground biodiversity and ecosystem functioning. Nature 515, 505–511.
Blagodatskaya, E., Kuzyakov, Y., 2013. Active microorganisms in soil: critical review of estimation criteria and approaches. Soil Biology and Biochemistry 67, 192–211.
Blasko, R., Bach, L.H., Yarwood, S.A., Trumbore, S.E., Hogberg, P., Hogberg, M.N., 2015. Shifts in soil microbial community structure, nitrogen cycling and the concomitant declining N availability in ageing primary boreal forest ecosystems. Soil Biology and Biochemistry 91, 200–211.
Bradford, M.A., Berg, B., Maynard, D.S., Wieder, W.R., Wood, S.A., 2016a. Understanding the dominant controls on litter decomposition. Journal of Ecology 104, 229–238.
Bradford, M.A., Wieder, W.R., Bonan, G.B., Fierer, N., Raymond, P.A., Crowther, T.W., 2016b. Managing uncertainty in soil carbon feedbacks to climate change. Nature Climate Change 6, 751–758.
Calder, F.W., Macleod, L.B., 1974. Effects of soil ph and Npk fertilization on yield and quality of 2 barley cultivars. Canadian Journal of Soil Science 54, 1–6.
Cruz-Paredes, C., Wallander, H., Kjoller, R., Rousk, J., 2017. Using community trait-distributions to assign microbial responses to pH changes and Cd in forest soils treated with wood ash. Soil Biology and Biochemistry 112, 153–164.
de Boer, W., Folman, L.B., Summerbell, R.C., Boddy, L., 2005. Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiology Reviews 29, 795–811.
de Vries, F.T., Hoffland, E., van Eekeren, N., Brussaard, L., Bloem, J., 2006. Fungal/bacterial ratios in grasslands with contrasting nitrogen management. Soil Biology and Biochemistry 38, 2092–2103.
DeForest, J.L., Zak, D.R., Pregitzer, K.S., Burton, A.J., 2004. Atmospheric nitrate deposition, microbial community composition, and enzyme activity in northern hardwood forests. Soil Science Society of America Journal 68, 132–138.
del Giorgio, P.A., Cole, J.J., 1998. Bacterial growth efficiency in natural aquatic systems. Annual Review of Ecology and Systematics 29, 503–541.
Denman, K.L., Brasseur, G., Chidthaisong, A., Ciais, P., Cox, P.M., Dickinson, R.E., Hauglustaine, D., Heinze, C., Holland, E., Jacob, D., Lohmann, U., Ramachandran, S., da Silva Dias, P.L., Wofsy, S.C., Zhang, X., 2007. Couplings between Changes in the Climate System and Biogeochemistry. Cambridge University Press, Cambridge, UK.
Dijkstra, P., Dalder, J.J., Selmants, P.C., Hart, S.C., Koch, G.W., Schwartz, E., Hungate, B.A., 2011. Modeling soil metabolic processes using isotopologue pairs of position-specific C-13-labeled glucose and pyruvate. Soil Biology and Biochemistry 43,

1848–1857.
Fernandez-Calvino, D., Bååth, E., 2010. Growth response of the bacterial community to pH in soils differing in pH. FEMS Microbiology Ecology 73, 149–156.
Fierer, N., Colman, B.P., Schimel, J.P., Jackson, R.B., 2006. Predicting the temperature dependence of microbial respiration in soil: a continental-scale analysis. Global Biogeochemical Cycles 20.
Fierer, N., Craine, J.M., McLauchlan, K., Schimel, J.P., 2005. Litter quality and the temperature sensitivity of decomposition. Ecology 86, 320–326.
Fog, K., 1988. The effect of added nitrogen on the rate of decomposition of organic-matter. Biological Reviews of the Cambridge Philosophical Society 63, 433–462.
Frey, S.D., Lee, J., Melillo, J.M., Six, J., 2013. The temperature response of soil microbial efficiency and its feedback to climate. Nature Climate Change 3, 395–398.
Geyer, K.M., Dijkstra, P., Sinsabaugh, R., Frey, S.D., 2019. Clarifying the interpretation of carbon use efficiency in soil through methods comparison. Soil Biology and Biochemistry 128, 79–88.
Geyer, K.M., Kyker-Snowman, E., Grandy, A.S., Frey, S.D., 2016. Microbial carbon use efficiency: accounting for population, community, and ecosystem-scale controls over the fate of metabolized organic matter. Biogeochemistry 127, 173–188.
Giesler, R., Hogberg, M., Hogberg, P., 1998. Soil chemistry and plants in Fennoscandian boreal forest as exemplified by a local gradient. Ecology 79, 119–137.
Henriksen, T.M., Breland, T.A., 1999. Nitrogen availability effects on carbon mineralization, fungal and bacterial growth, and enzyme activities during decomposition of wheat straw in soil. Soil Biology and Biochemistry 31, 1121–1134.
Jansa, J., Mozafar, A., Kuhn, G., Anken, T., Ruh, R., Sanders, I.R., Frossard, E., 2003. Soil tillage affects the community structure of mycorrhizal fungi in maize roots. Ecological Applications 13, 1164–1176.
Johnson, D., Leake, J.R., Read, D.J., 2005. Liming and nitrogen fertilization affects phosphatase activities, microbial biomass and mycorrhizal colonisation in upland grassland. Plant and Soil 271, 157–164.
Jones, D.L., Willett, V.B., 2006. Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. Soil Biology and Biochemistry 38, 991–999.
Jörgensen, R.G., 2000. Ergosterol and microbial biomass in the rhizosphere of grassland soils. Soil Biology and Biochemistry 32, 647–652.
Kallenbach, C.M., Frey, S.D., Grandy, A.S., 2016. Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. Nature Communications 7.
Kristensen, J.A., Metcalfe, D.B., Rousk, J., 2018. The biogeochemical consequences of litter transformation by insect herbivory in the Subarctic: a microcosm simulation experiment. Biogeochemistry 138, 323–336.
Lange, M., Eisenhauer, N., Sierra, C.A., Bessler, H., Engels, C., Griffiths, R.I., Mellado-Vazquez, P.G., Malik, A.A., Roy, J., Scheu, S., Steinbeiss, S., Thomson, B.C., Trumbore, S.E., Gleixner, G., 2015. Plant diversity increases soil microbial activity and soil carbon storage. Nature Communications 6.
Le Guillou, C., Angers, D.A., Maron, P.A., Leterme, P., Menasseri-Aubry, S., 2012. Linking microbial community to soil water-stable aggregation during crop residue decomposition. Soil Biology and Biochemistry 50, 126–133.
Liang, C., Schimel, J.P., Jastrow, J.D., 2017. The importance of anabolism in microbial control over soil carbon storage. Nature Microbiology 2.
Lucas, S.T., D'Angelo, E.M., Williams, M.A., 2014. Improving soil structure by promoting fungal abundance with organic soil amendments. Applied Soil Ecology 75, 13–23.
Malik, A.A., Chowdhury, S., Schlager, V., Oliver, A., Puissant, J., Vazquez, P.G.M., Jehmlich, N., von Bergen, M., Griffiths, R.I., Gleixner, G., 2016. Soil fungal:bacterial ratios are linked to altered carbon cycling. Frontiers in Microbiology 7.
Manzoni, S., Taylor, P., Richter, A., Porporato, A., Agren, G.I., 2012. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. New Phytologist 196, 79–91.
Newell, S.Y., Fallon, R.D., 1991. Toward a method for measuring instantaneous fungal growth-rates in field samples. Ecology 72, 1547–1559.
Ramirez, K.S., Craine, J.M., Fierer, N., 2010. Nitrogen fertilization inhibits soil microbial respiration regardless of the form of nitrogen applied. Soil Biology and Biochemistry 42, 2336–2338.
Roller, B.R.K., Schmidt, T.M., 2015. The physiology and ecological implications of efficient growth. The ISME Journal 9, 1481–1487.
Rosinger, C., Rousk, J., Sanden, H., 2019. Can enzymatic stoichiometry be used to determine growth-limiting nutrients for microorganisms? - a critical assessment in two subtropical soils. Soil Biology and Biochemistry 128, 115–126.
Rousk, J., 2016. Biomass or growth? How to measure soil food webs to understand structure and function. Soil Biology and Biochemistry 102, 45–47.
Rousk, J., Bååth, E., 2007a. Fungal and bacterial growth in soil with plant materials of different C/N ratios. FEMS Microbiology Ecology 62, 258–267.
Rousk, J., Bååth, E., 2007b. Fungal biomass production and turnover in soil estimated using the acetate-in-ergosterol technique. Soil Biology and Biochemistry 39, 2173–2177.
Rousk, J., Brookes, P.C., Bååth, E., 2011. Fungal and bacterial growth responses to N fertilization and pH in the 150-year 'Park Grass' UK grassland experiment. FEMS Microbiology Ecology 76, 89–99.
Rousk, J., Brookes, P.C., Bååth, E., 2009. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. Applied and Environmental Microbiology 75, 1589–1596.
Rousk, J., Brookes, P.C., Bååth, E., 2010. Investigating the mechanisms for the opposing pH relationships of fungal and bacterial growth in soil. Soil Biology and Biochemistry 42, 926–934.
Rousk, J., Demoling, L.A., Bahr, A., Bååth, E., 2008. Examining the fungal and bacterial niche overlap using selective inhibitors in soil. FEMS Microbiology Ecology 63, 350–358.
Rousk, J., Frey, S.D., 2015. Revisiting the hypothesis that fungal-to-bacterial dominance characterizes turnover of soil organic matter and nutrients. Ecological Monographs 85, 457–472.
Rousk, K., Rousk, J., Jones, D.L., Zackrisson, O., DeLuca, T.H., 2013. Feather moss nitrogen acquisition across natural fertility gradients in boreal forests. Soil Biology and Biochemistry 61, 86–95.
Sinsabaugh, R.L., Manzoni, S., Moorhead, D.L., Richter, A., 2013. Carbon use efficiency of microbial communities: stoichiometry, methodology and modelling. Ecology Letters 16, 930–939.
Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Science Society of America Journal 70, 555–569.
Soares, M., Rousk, J., 2019. Microbial growth and carbon use efficiency in soil: links to fungal-bacterial dominance, SOC-quality and stoichiometry. Soil Biology and Biochemistry 131, 195–205.
Spohn, M., Klaus, K., Wanek, W., Richter, A., 2016. Microbial carbon use efficiency and biomass turnover times depending on soil depth - implications for carbon cycling. Soil Biology and Biochemistry 96, 74–81.
Sterkenburg, E., Bahr, A., Durling, M.B., Clemmensen, K.E., Lindahl, B.D., 2015. Changes in fungal communities along a boreal forest soil fertility gradient. New Phytologist 207, 1145–1158.
Sterner, R.W., Elser, J.J., 2002. Stoichiometry in Microbial Communities: Dynamics and Interactions. Princeton University Press, Princeton, NJ, USA.
Strickland, M.S., Osburn, E., Lauber, C., Fierer, N., Bradford, M.A., 2009. Litter quality is in the eye of the beholder: initial decomposition rates as a function of inoculum characteristics. Functional Ecology 23, 627–636.
Strickland, M.S., Rousk, J., 2010. Considering fungal:bacterial dominance in soils - methods, controls, and ecosystem implications. Soil Biology and Biochemistry 42, 1385–1395.
Waksman, S.A., Starkey, R.L., 1924. Microbiological analysis of soil as an index of soil fertility. Soil Science 17, 141–161.
Walker, T.W.N., Kaiser, C., Strasser, F., Herbold, C.W., Leblans, N.I.W., Woebken, D., Janssens, I.A., Sigurdsson, B.D., Richter, A., 2018. Microbial temperature sensitivity and biomass change explain soil carbon loss with warming (vol 8, pg 885, 2018). Nature Climate Change 8 1021-1021.
Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setala, H., van der Putten, W.H., Wall, D.H., 2004. Ecological linkages between aboveground and belowground biota. Science 304, 1629–1633.
Whalen, E.D., Smith, R.G., Grandy, A.S., Frey, S.D., 2018. Manganese limitation as a mechanism for reduced decomposition in soils under atmospheric nitrogen deposition. Soil Biology and Biochemistry 127, 252–263.
WRB, I.W.G., 2015. World Reference Base for Soil Resources 2014, update 2015 International soil classification system for naming soils and creating legends for soil maps. World Soil Resources Reports.
Zheng, Q., Hu, Y.T., Zhang, S.S., Noll, L., Bockle, T., Richter, A., Wanek, W., 2019. Growth explains microbial carbon use efficiency across soils differing in land use and geology. Soil Biology and Biochemistry 128, 45–55.